Supporting Information for

Tuning Hydrogel Properties and Function Using Substituent Effects

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General:

Reactions were monitored by thin layer chromatography (TLC) using silica gel 60 F_{254} plastic, aluminum, or glass plates (Aldrich, Merck or EMD Chemicals, Inc.) or F_{264} basic aluminum oxide glass plates (EMD Chemicals, Inc.). Gravity and flash chromatography was performed with 32-63 micron silica gel (Merck). A fluorescent indicator (green 254 nm) was added to the silica gel for chromatography performed in a quartz column. TLC bands were visualized by UV, dinitrophenylhydrazine (DNP) stain, or ninhydrin stain. Solvent ratios used as elutants are reported in v/v. The purity of the final products was obtained through ^{1}H NMR, ^{13}C NMR, and/or elemental analysis.

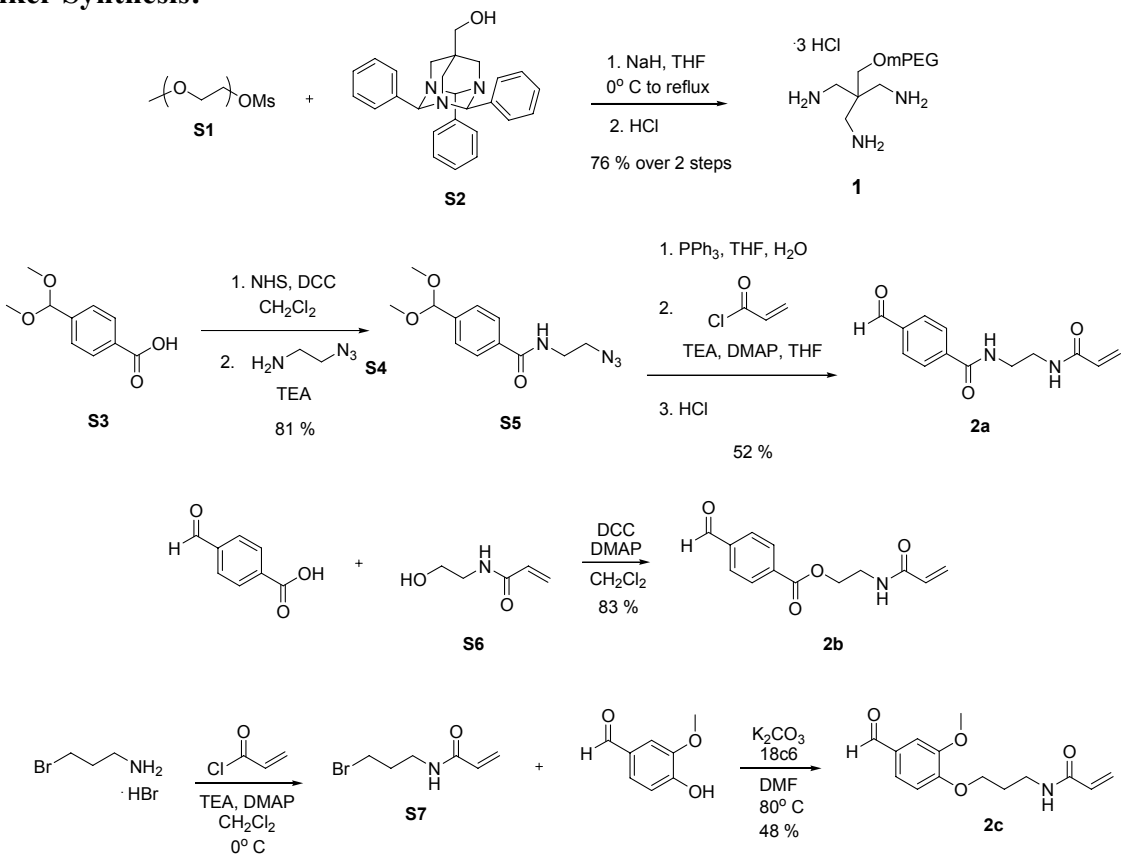
^{1}H NMR and ^{13}C NMR data were obtained on either a 400 or 500 MHz Varian U400 or U500 instrument at the VOICE NMR laboratory at the University of Illinois. Chemical shifts were reported in parts per million (ppm), and coupling constants were reported in Hertz (Hz). ^{1}H NMR spectra obtained in $CDCl_3$ were referenced to 7.26 ppm, D_2O were referenced to 4.79 ppm, and DMSO-d_6 were referenced to 2.50 ppm. ^{13}C NMR spectra obtained in $CDCl_3$ were referenced to 77.16 ppm. Electro-spray ionization mass spectrometry (ESIMS) data were collected by the Mass Spectrometry Center at the University of Illinois. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) spectra were

obtained on an Applied Biosystems Voyager-DE STR mass spectrometer. Matrices employed in the collection of MALDI spectra included dithranol, 2-(4-hydroxyphenylazo)benzoic acid (HABA), and trans-3-indoleacrylic acid (IAA). Data were calibrated to an external standard solution of insulin and cytochrome C. Infrared spectra (IR) were collected on a Matson FTIR 5000. Circular dichroism spectra were collected on a Jasko J-720 spectropolarimeter.

BSA (albumin from bovine serum, minimum 98% elecrophoresis) and polyethyleneimine (branched 25 kDa) were purchased from Sigma and used with no further purification. The following compounds were synthesized according to literature procedures: mPEG750-OMs (**S1**),[1] 7-(hydroxymethyl)-2,4,6-triphenyl-1,3,5-triazaadamantane (**S2**),[2] 4-carboxybenzaldehyde dimethoxyacetal (**S3**),[3] 1-azido-2-aminoethane (**S4**),[4] and N-(2-hydroxyethyl)acrylamide (**S6**).[5] Compounds characterized by ^{1}H NMR spectroscopy.

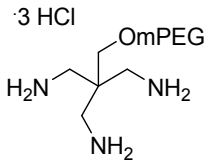
Experiments with live animals involved the use of chick embryo chorioallantoic membrane (CAM), which do not require approval from the Institutional Animal Care and Use Committee (IACUC).

Cross-linker Synthesis:

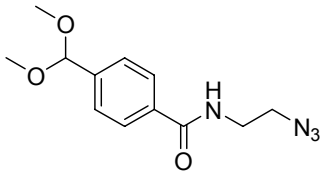


Scheme S1. Synthesis of compounds **1** and **2a-c**.

Synthesis and Characterization:

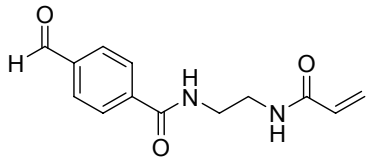


2-Polyethyleneglycol methyl ether-1,1,1-tris(aminomethyl)ethane trihydrochloride (1). To a solution of 12.6 g (31.7 mmol) of 7-(hydroxymethyl)-2,4,6-triphenyl-1,3,5-triazaadamantane (**S2**) in 100 mL of THF at 0˚C was added 6.24 g (156 mmol) of sodium hydride and the solution was stirred for 1 h. 17.5 g (21.2 mmol) of mPEG750-OMs (**S1**) was added and the solution was heated to reflux for 20 h. 5 mL of MeOH was added and the solution was concentrated in vacuo and partitioned between 500 mL of CH_2Cl_2 and 100 mL of brine. MeOH was added to the resulting emulsion until the separate layers were observed. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. MeOH was added to the residue and filtered to remove insoluble materials. Concentration in vacuo produced 30.0 g of an orange oil that was stirred in HCl (1 M) for 30 min. The solution was washed once with 300 mL of ether and twice with 100 mL of ether and concentrated in vacuo. CH_2Cl_2 was added, insoluble material was filtered, and the solution was concentrated in vacuo to produce 15.6 g (76 % over 2 steps) of **1** as a brown paste: ^{1}H NMR (500 MHz, D_2O) δ 3.84-3.63 (br m, ~66H), 3.42 (s, 3H), 3.35 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 72.3, 71.7, 70.3, 61.4, 58.8; MALDI-TOF LRMS indicates product distribution centered around *m/z* 918.4 ($M + Na^+$).

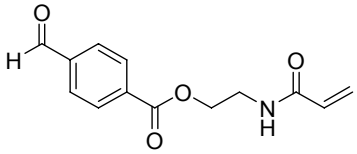


N-(2-Azido-ethyl)-4-dimethoxymethyl-benzamide (S5). To a solution of 13.4 g (68.3 mmol) of 4-carboxybenzaldehyde dimethoxyacetal (**S3**) and 8.81 g (75.1 mmol) of N-hydroxysuccinimide in 300 mL of CH_2Cl_2 was added dropwise 15.0 g (71.7 mmol) of N,N'-dicyclohexylcarbodiimide in 40 mL of CH_2Cl_2 over 30 min and stirred for an additional 1.25 h. The precipitate was filtered and solvent was

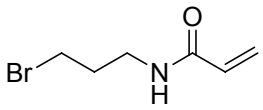
reduced in vacuo to a total of approximately 100 mL. 7.27 g (84.5 mmol) of 1-azido-2-aminoethane (**S4**), 12.0 mL (86.1 mmol) of triethylamine, and 415 mg (3.36 mmol) of 4-(dimethylamino)pyridine were added and the solution was stirred for 2.25 h. The solution was washed twice with 100 mL of $NaHCO_3$, dried over Na_2SO_4, filtered, concentrated in vacuo, and purified via flash chromatography (1:1 ethyl acetate : petroleum ether) to afford 14.6 g (81 %) of **S5** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 6.43 (br s, 2H), 5.44 (s, 1H), 3.65 (q, *J* = 5.6 Hz, 2H), 3.58 (t, *J* = 5.6 Hz, 2H), 3.32 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.8, 141.7, 134.1, 127.0, 127.0, 102.3, 52.7, 50.7, 39.5; ESI-LRMS *m/z* 265.4 (M^+).



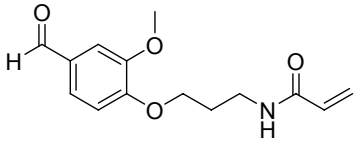
Substituted benzaldehyde 2a. To a solution of 14.3 g (54.0 mmol) of **S5** in 100 mL of THF was added 16.9 g (63.9 mmol) of triphenylphosphine and the solution was stirred for 5 h. 5 mL of H_2O was added and the solution was stirred for 25 h. 15.0 mL (108 mmol) of triethylamine and 339 mg (2.75 mmol) of 4-(dimethylamino)pyridine were added and this solution was added dropwise over 1 h to a solution of 9.00 mL (106 mmol) of acryloyl chloride in 30 mL of THF at 0° C and the resulting solution was stirred for 4 h. Insoluble material was filtered, the solution was reduced in vacuo, 100 mL of HCl (1M) and CH_2Cl_2 was added. The organic layer was washed with 100 mL of HCl (1M), dried over Na_2SO_4, filtered, concentrated in vacuo, and purified via flash chromatography (gradient 5% to 6% MeOH in CH_2Cl_2) to afford 6.93 g (52 %) of **2a** as an off-white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.1 (s, 1H), 7.98 (d, *J* = 8.0 Hz, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.64 (br s, 1H), 6.33 (br s, 1H), 6.33 (d, *J* = 16.7 Hz, 1H), 6.13 (dd, *J* = 10.2 Hz, 16.7 Hz, 1H), 5.70 (d, *J* = 10.2 Hz, 1H), 3.63 (br s, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9, 167.8, 167.1, 139.3, 138.4, 130.4, 130.0, 127.9, 127.7, 42.2, 39.9; ESI-LRMS *m/z* 247.2 (M^+); Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.30. Found: C, 63.24; H, 5.69; N, 11.16.



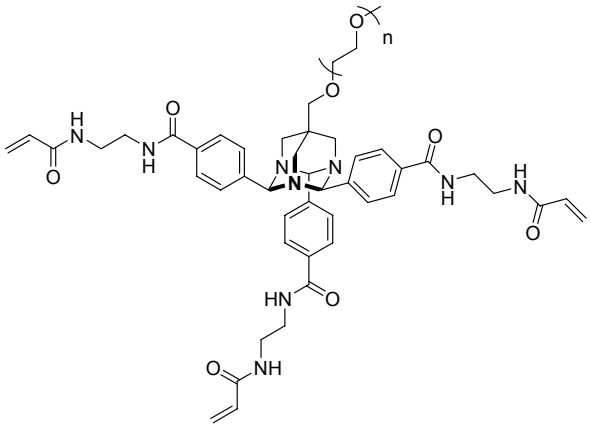
Substituted benzaldehyde 2b. To a suspension of 4.63 g (30.2 mmol) of 4-carboxybenzaldehyde, 4.18 g (36.3 mmol) of N-(2-hydroxyethyl)acrylamide (**S6**), and 379 mg (3.07 mmol) of 4-(dimethylamino)pyridine in 120 mL of CH_2Cl_2 was added dropwise 6.98 g (33.5 mmol) of N,N'-dicyclohexylcarbodiimide in 50 mL of CH_2Cl_2 over 2.25 h and the resulting solution was stirred for 10 h. The reaction was filtered, concentrated in vacuo, and purified via flash chromatography (gradient 2:1 EtOAc : pet ether to EtOAc) to afford 6.20 g (83 %) of **2b** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.1 (s, 1H), 8.20 (d, *J* = 8.2 Hz, 2H), 7.97 (d, *J* = 8.2 Hz, 2H), 6.31 (dd, *J* = 1.3 Hz, 16.9 Hz, 1H), 6.12 (dd, *J* = 10.2 Hz, 16.9 Hz, 1H), 5.95 (br s, 1H), 5.68 (dd, *J* = 1.3 Hz, 10.2 Hz, 1H), 4.51 (t, *J* = 5.4 Hz, 2H), 3.78 (q, *J* = 5.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.8, 166.0, 165.7, 139.3, 134.8, 130.6, 130.4, 129.6, 127.1, 64.3, 38.8; ESI-LRMS *m/z* 248.3 (M^+); Anal. Calcd for $C_{13}H_{13}NO_4$: C, 63.14; H, 5.30; N, 5.67. Found: C, 63.02; H, 5.25; N, 5.90.



***N*-(3-Bromo-propyl)-acrylamide (S7).** To a solution of 4.20 mL (49.6 mmol) of acryloyl chloride in 50 mL of CH_2Cl_2 at 0° C was added dropwise a solution of 10.1 g (45.3 mmol) of 3-bromopropylamine hydrobromide, 14.0 mL (100 mL) of triethylamine, and 288 mg (2.34 mmol) of 4-(dimethylamino)pyridine in 140 mL of CH_2Cl_2 and the resulting solution was stirred for 4.5 h. The solution was concentrated in vacuo, diluted with ethyl acetate, and filtered. 6.3 mg (1.4 mmol) of 2,6-bis(1,1-dimethylethyl)-4-methylphenol was added and the solution was concentrated in vacuo to afford 10.6 g of **S7** as a yellow liquid that was taken forward without further purification.

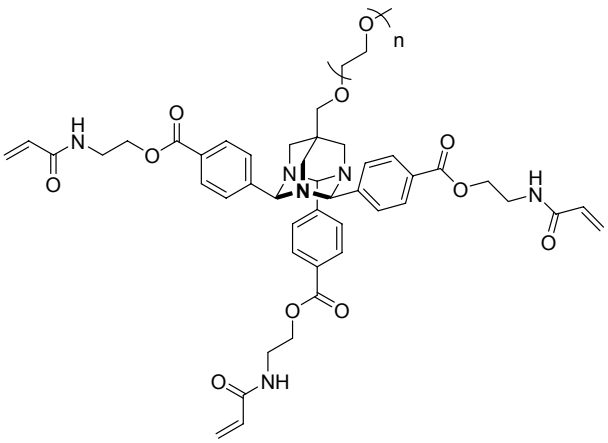


Substituted benzaldehyde 2c. To a suspension of 9.12 g (66.0 mmol) of potassium carbonate, 4.91 g (32.2 mmol) of vanillin, and 488 mg (1.83 mmol) of 18-crown-6 stirring in 50 mL of DMF at 80 °C was added 8.70 g, (45.3 mmol; theoretical from previous) of **S7** in 50 mL of DMF in five equal portions each hour over 5 h and stirred for an additional 2 hours after the final addition. The reaction was cooled to room temperature, filtered, and reduced in vacuo. The crude product was dissolved in ethyl acetate, filtered, reduced in vacuo, and purified via flash chromatography (gradient EtOAc to 10% MeOH in EtOAc) to afford 4.09 g (48 %) of **2c** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.87 (s, 1H), 7.46 (dd, *J* = 1.8 Hz, 8.2 Hz, 1H), 7.44 (d, *J* = 1.8 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 6.71 (br s, 1H), 6.31 (dd, *J* = 1.5 Hz, 17.1 Hz, 1H), 6.12 (dd, *J* = 10.3 Hz, 17.1 Hz, 1H), 5.66 (dd, *J* = 1.5 Hz, 10.2 Hz, 1H), 4.24 (t, *J* = 5.7 Hz, 2H), 3.96 (s, 3H), 3.62 (quart, *J* = 5.7 Hz, 2H), 2.13 (quint, *J* = 5.7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.8, 165.6, 153.4, 149.6, 131.1, 130.2, 126.9, 126.0, 111.2, 109.0, 68.5, 55.9, 37.8, 28.5; ESI-LRMS *m/z* 264.4 (M^+); Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.86; H, 6.56; N, 5.38.



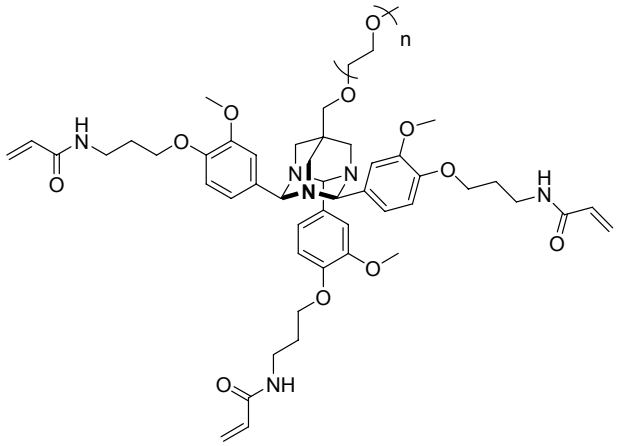
Amide Substituted Triazaadamantane (3a). To a solution of 1.37 g (1.41 mmol) of 2-polyethyleneglycol methyl ether-1,1,1-tris(aminomethyl)ethane trihydrochloride **1** and 1.00 mL (7.17 mmol) of triethylamine in 30 mL of MeOH was added 1.18 g (4.77 mmol) of substituted benzaldehyde **2a** and the solution was refluxed for 1 h. The solution was concentrated in vacuo and purified via flash

chromatography (gradient 10 % MeOH, 0.5 % NH_4OH in CH_2Cl_2 to 16 % MeOH, 0.5 % NH_4OH in CH_2Cl_2). The product was further partitioned between 100 mL CH_2Cl_2 and 50 mL $NaHCO_3$. The aqueous layer was washed with 50 mL CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 1.53 g (70 %) of **3a** as a clear, pale tan oil. Major diastereomer: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.60 (t, *J* = 5.3 Hz, 2H), 8.49 (t, *J* = 5.3 Hz, 1H), 8.26 (t, *J* = 5.4 Hz, 2H), 8.19 (t, *J* = 5.4 Hz, 1H), 7.95 (d, *J* = 8.3 Hz, 4H), 7.87 (d, *J* = 8.5 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.2 Hz, 4H), 6.25-6.13 (m, 3H), 6.10 (dd, *J* = 2.2 Hz, 17.1 Hz, 2H), 6.06 (dd, *J* = 2.2 Hz, 17.2 Hz, 1H), 5.77 (s, 1H), 5.60 (dd, *J* = 2.2 Hz, 10.2 Hz, 2H), 5.56 (dd, *J* = 2.2 Hz, 10.2 Hz, 1H), 5.26 (s, 2H), 3.23 (s, 3H), 3.47-3.24 (br m, PEG region +16H), 2.86 (d, *J* = 10.6 Hz, 2H), 2.73 (s, 2H); MALDI-TOF LRMS indicates product distribution centered around *m/z* 1647.1 ($M + Na^+$).



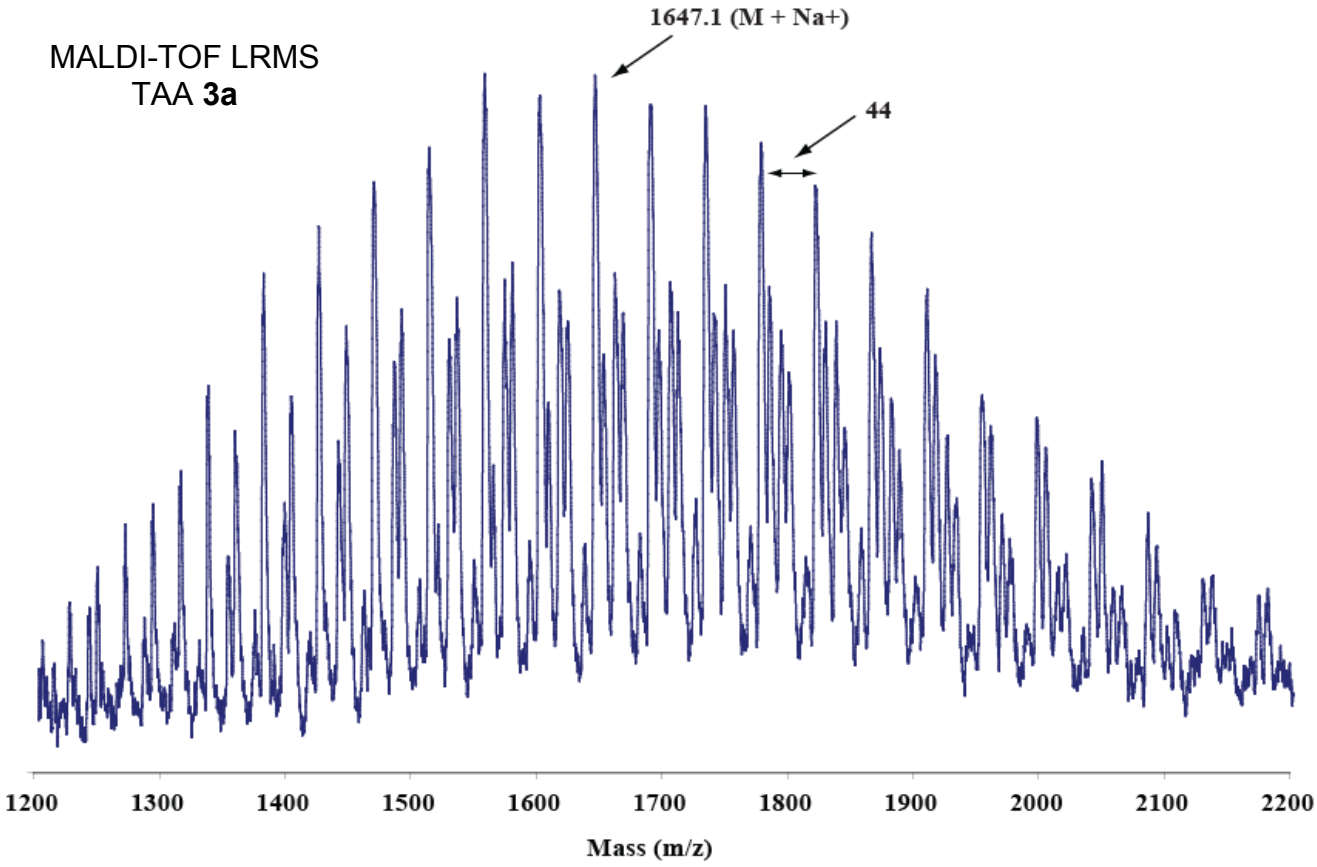
Ester Substituted Triazaadamantane (3b). To a solution of 1.32 g (1.35 mmol) of 2-polyethyleneglycol methyl ether-1,1,1-tris(aminomethyl)ethane trihydrochloride **1** and 1.00 mL (7.17 mmol) of triethylamine in 30 mL of MeOH was added 1.14 g (4.62 mmol) of substituted benzaldehyde **2b** and the solution was refluxed for 1 h. Insoluble material was filtered and the solution was concentrated in vacuo. EtOAc was added and the insoluble material was filtered. The solution was concentrated in vacuo and purified via flash chromatography (gradient 10 % MeOH, 0.5 % NH_4OH in CH_2Cl_2 to 12 % MeOH, 0.5 % NH_4OH in CH_2Cl_2) to afford 1.28 g (61 %) of **3b** as a clear, pale tan oil. Major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.3 Hz, 4H), 8.04 (d, *J* = 8.3 Hz, 2H), 7.90 (d, *J* = 8.3 Hz, 2H), 7.83 (d, *J* = 8.3 Hz, 4H), 6.37-6.00 (m, 9H), 5.67 (dd, *J* = 1.3 Hz, 10.1 Hz, 2H),

5.65 (s, 1H), 5.62 (dd, *J* = 1.3 Hz, 10.1 Hz, 1H), 5.34 (s, 2H), 4.49 (t, *J* = 5.2 Hz, 4H), 4.43 (t, *J* = 5.2 Hz, 2H), 3.77 (quart, *J* = 5.4 Hz, 4H), 3.71 (quart, *J* = 5.4 Hz, 2H), 3.68-3.39 (br m, PEG region +4H), 3.37 (s, 3H), 3.32 (t, , *J* = 4.7 Hz, 2H), 3.24 (d, *J* = 12.7 Hz, 2H), 2.88 (s, 2H), 2.72 (s, 2H); MALDI-TOF LRMS indicates product distribution centered around *m/z* 1606.3 ($M + Na^+$).



Ether Substituted Triazaadamantane (3c). To a solution of 1.34 g (1.37 mmol) of 2-polyethyleneglycol methyl ether-1,1,1-tris(aminomethyl)ethane trihydrochloride **1** and 1.00 mL (7.17 mmol) triethylamine in 40 mL of MeOH was added 1.23 g (4.67 mmol) of substituted benzaldehyde **2c** and the solution was refluxed for 1 h. The solution was concentrated in vacuo and purified via flash chromatography (8 % MeOH, 0.5 % NH_4OH in CH_2Cl_2) to afford 1.59 g (73 %) of **3c** as a clear, pale tan oil. Major diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.33 (s, 2H), 7.31-7.25 (m, 3H), 7.00 (t, *J* = 5.2 Hz, 2H), 6.97-6.89 (m, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.33 (dd, *J* = 1.5 Hz, 17.1 Hz, 2H), 6.27 (dd, *J* = 1.5 Hz, 17.1 Hz, 1H), 6.16 (dd, *J* = 10.1 Hz, 17.1 Hz, 2H), 6.09 (dd, *J* = 10.1 Hz, 17.1 Hz, 1H), 5.65 (dd, *J* = 1.5 Hz, 10.1 Hz, 2H), 5.59 (dd, *J* = 1.5 Hz, 10.1 Hz, 1H), 5.56 (s, 1H), 5.36 (s, 2H), 4.19 (t, *J* = 5.4 Hz, 4H), 4.11 (t, *J* = 5.4 Hz, 2H), 3.94 (s, 6H), 3.85 (s, 3H), 3.70-3.45 (br m, PEG region +8H), 3.38 (s, 3H), 3.36 (t, *J* = 4.7 Hz, 2H), 3.23 (d, *J* = 12.7 Hz, 2H), 2.91 (s, 2H), 2.74 (s, 2H), 2.10 (quint, *J* = 5.6 Hz, 4H), 2.03 (quint, *J* = 5.6 Hz, 2H); MALDI-TOF LRMS indicates product distribution centered around *m/z* 1589.1 ($M + H^+$).

Spectra:


MALDI-TOF LRMS
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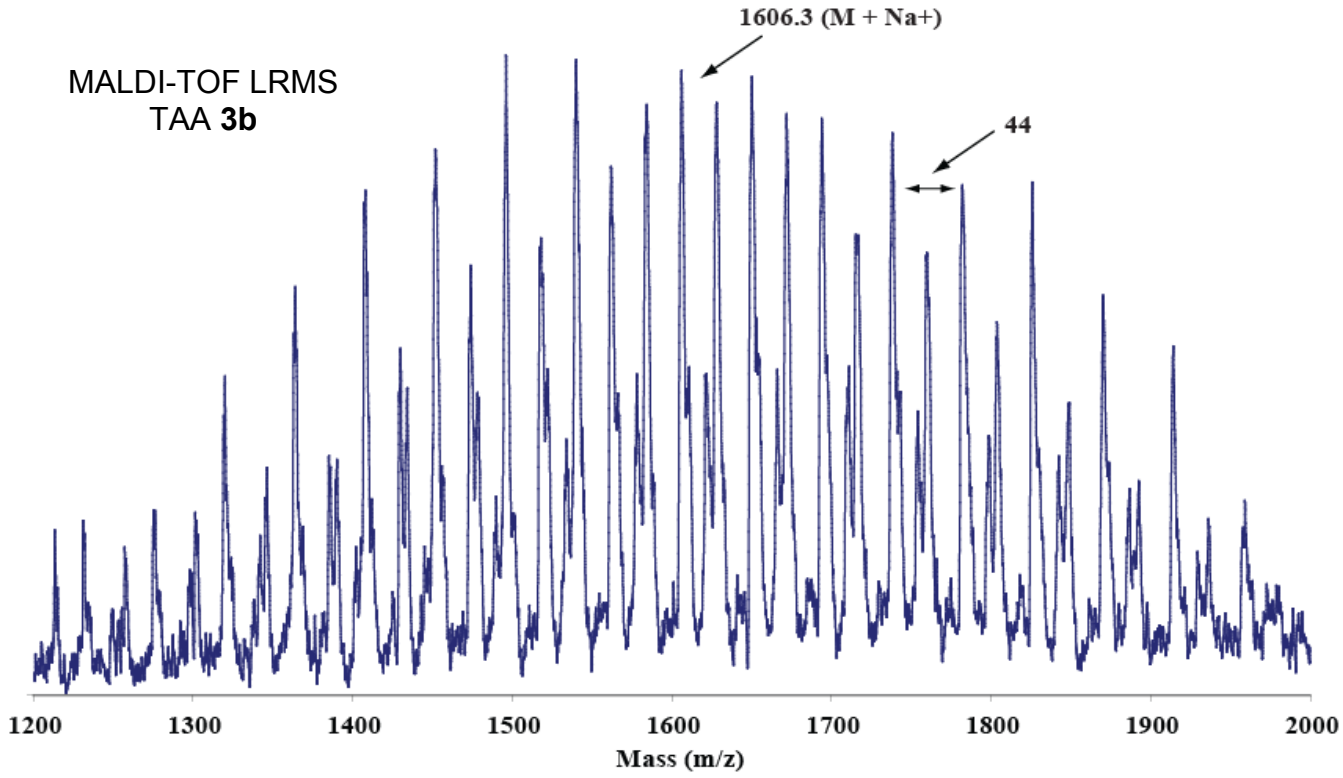
Mass (m/z)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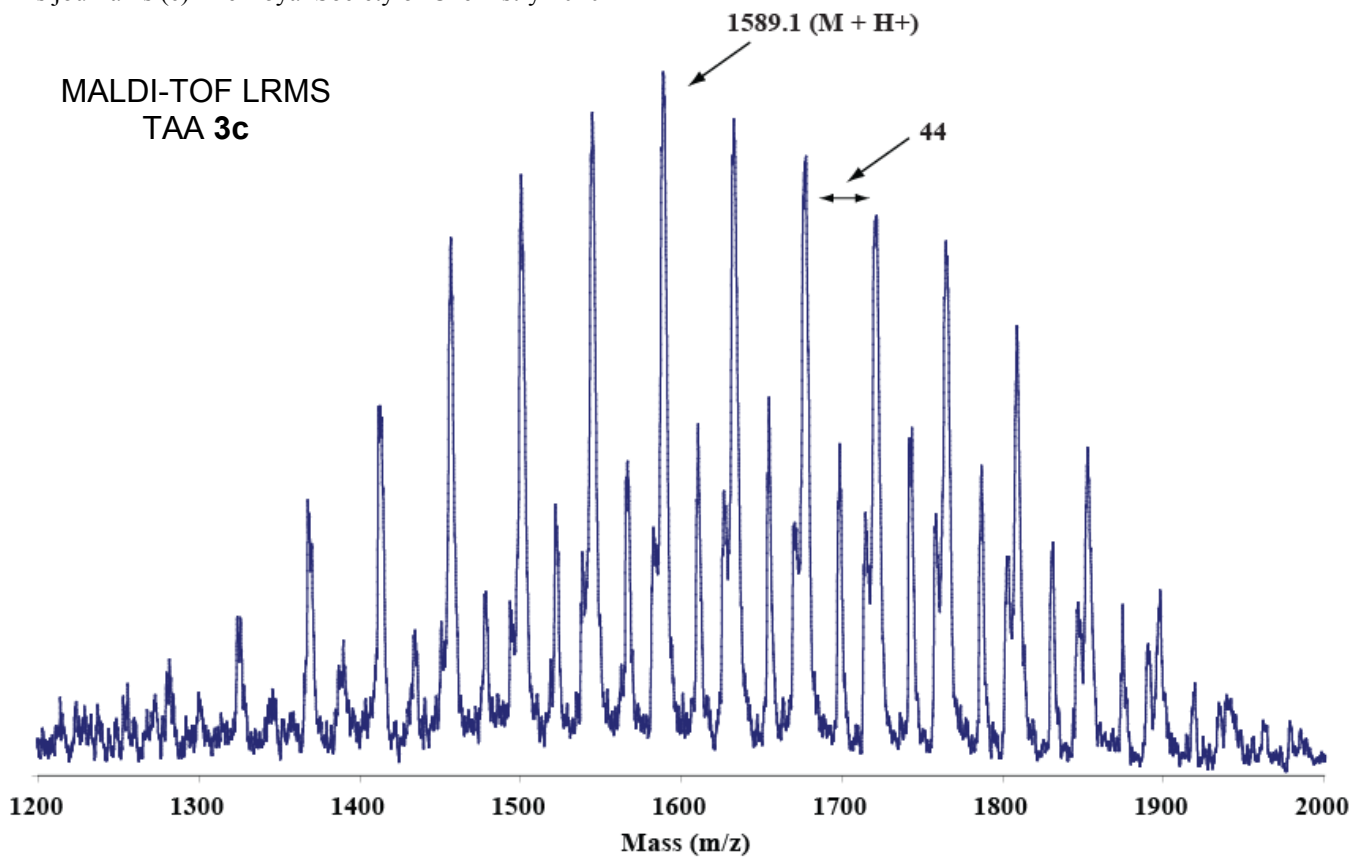
Mass (m/z)



General Hydrogel Syntheses:

A solution of a given monomer was dissolved in water to the desired concentration (wt %) and added to a vial containing **3a**, **3b**, or **3c** at an amount that gives the desired molar ratio of monomer to crosslinker. 0.5 wt % 2-hydroxy-2-methyl propiophenone was added and the solution was sterilized via filtration if necessary. The solution was placed in between 2 glass slides separated by 1 mm thick microscope slides and irradiated at 366 nm for 5 minutes using a hand held UV lamp (Entela, model UVGL-58). Hydrogel discs were made using a 10 mm diameter punch.

Elastic Modulus Determination:

Poly(acrylamide) hydrogel discs were made using the general synthesis method. 10 wt % acrylamide solutions were used for all studies. For cross-linkers **3a** and **3b,** a 1:99 mole ratio of crosslinker:acrylamide was used. For **3c,** a by mole ratio of 1:99, 1:299, and 1:900 was used. All hydrogel discs were incubated in deionized water at 37 °C for 40 minutes and the elastic modulus was measured using a 1 kN MTS Insight Electromechanical Testing System at a constant dformation rate of 1

mm/min at 25 °C. The compressive elastic moduli of the discs were calculated from the slope of a stress (σ) versus strain (λ) curve at the first 10% strain.

In vitro Degradation Study:

Poly(acrylamide) hydrogel discs were made using the general synthesis method. 9 wt % acrylamide solutions were used for all studies. For cross-linkers **3a** and **3b**, a 1:99 mole ratio of crosslinker:acrylamide was used. For **3c**, a mole ratio of 1:99, 1:299, and 1:900 was used. Hydrogel discs were incubated in 100 mM PBS pH 5.0 buffer, 100 mM PBS pH 7.4 buffer, or 100 mM carbonate pH 10.0 buffer at room temperature of 37 °C. Over time, the hydrogel disc diameter was measured to the nearest millimeter using a ruler and converted to surface area. Reported data points are an average of two experiments with the exception of the degradation study of discs made from **3c** incubated at pH 5.0 at 37 °C which is the average of four experiments.

In vivo Study Using Chick Embryo Chorioallantoic Membrane:

10 wt % acrylamide solutions were used for all studies. A 1:99 mole ratio of crosslinker:acrylamide was used for all studies. 0.7 wt % Irgacure 2959 was added and solutions were filtered through 0.2 μm filters. Solutions were placed between glass plates and irradiated at 366 nm for 10 minutes. Four discs were made using **3a**, five were made from **3b**, and five were made from **3c**. All discs were punched at a diameter of 5 mm. Discs were incubated in ethanol at 37 °C for 1 h followed by sterile PBS pH 7.4 for 19 h. The ethanol and buffer were each exchanged once during incubation. Discs were implanted in 11 day old chick eggs that had been opened 3 days before. Eggs were incubated at 37 °C for 4 more days after which formalin was added. Chorioallantoic membranes were cut out after 24 h, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Protein Release Study:

The general hydrogel syntheses procedure was used. Pre-gel solutions were made to contain 0.5 % Bovine Serum Albumin and 15 % acrylamide in PBS (100 mM, pH 7.4). A molar ratio of 1:19 TAA:acrylamide was used. Ten 5 mm discs were made using TAA **3a**; five were incubated at pH 7.4 (100mM PBS) and five at pH 5.0 (100 mM PBS). Discs were incubated in a 24 well plate with one disc per well submerged in 1 mL buffer. Nine discs were made using TAA **3c**; four were incubated at pH 7.4 and five at pH 5.0. At each time point 20 μL samples were removed for analysis and replaced by an equal volume of appropriate buffer. BSA amount was calculated using a commercially available BCA protein assay kit (Pierce). BSA containing samples were mixed with assay solution and the aliquot absorbances were compared to a BSA standard curve. The experiment was conducted in a 96 well plate using a spectrophotometer (Synergy HT, Biotek). Percent protein release was calculated by dividing the mass of protein released at a given time versus the theoretical amount of protein encapsulated. The theoretical amount of protein per disc was calculated by multiplying the concentration of the pre-gel solution (as determined by the BCA assay) by the volume of hydrogel disc (as calculated by the measured dimensions 5 mm diameter and 1 mm thickness).

Protein denaturation evaluation:

Four 5 mm diameter BSA containing dics were prepared using cross-linker **3c** and the Protein Release Study protocol. The discs were incubated at 37 °C and pH 5.0 (100 mM PBS). Discs were incubated in a 24 well plate with one disc per well submerged in 1 mL buffer. After 46 h the BSA concentration was determined using a BCA protein assay kit. The remaining solution was removed from the well, washed once with an equal volume of methylene chloride to remove chromophoric degradation products, and analyzed using circular dichroism. The spectrum was compared to solutions of BSA made from commercially available protein at pH 7.4 and 5.0 (100 mM).

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Assay:

MTT assays were employed to test the biocompatibility of the hydrogel degradation products. Each 5 mm diameter hydrogel disc (the same formulation used in degradation studies) was placed in a highly acidic solution (pH 1) for the complete degradation. The degraded products were collected by lyophilizing the acidic solution. Then, it was re-dissolved in 1 mL of cell growth medium (Dulbecco's Modified Eagle Medium (DMEM) containing 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin, all from ATCC) and the pH was adjusted to 7.4. 0.1 mL of the resulting solution was placed in a well of 96-well plate seeded with NIH3T3 fibroblasts (ATCC) at a cell density of 5000 cells/well. After 48 hours of incubation at 37°C with 5% atmospheric CO_2, 0.01 mL of MTT reagent (ATCC) was added and further incubated for 4 hours. Then, 0.1 mL of MTT detergent reagent (ATCC) was added and the well plate was shaken at room temperature under dark for 2 hours to dissolve the MTT product made by the viable cells. The amount of viable cells was quantified by measuring the absorbance of the MTT product at 570 nm using a spectrophotometer (Synergy HT, Biotek). 4 hydrogel disks were tested for each condition to obtain the average and standard deviation. MTT assay performed on pure media and the cells without the degradation product was taken as 0 % and 100 % viabilities, respectively. The MTT assay was performed with varying concentrations of the degraded product. Also the assay was performed for polyethyleneimine at the same concentrations as those used for the gel degradation products.

Supplemental Figures:

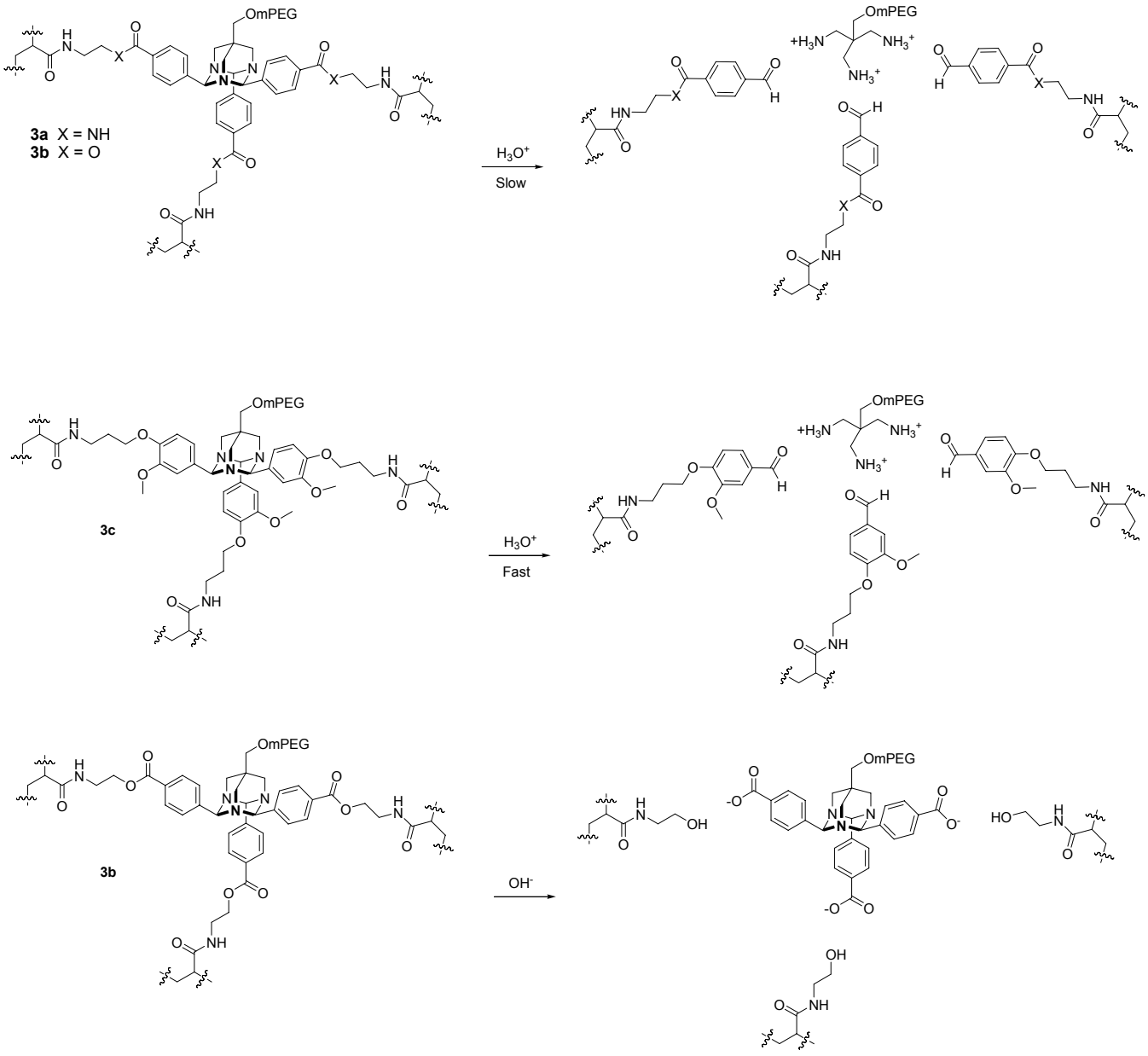


Fig. S1 Depiction of TAA crosslinked poly(acrylamide) degradation mechanisms under acid and basic conditions using cross-linkers **3a**, **3b**, or **3c**.

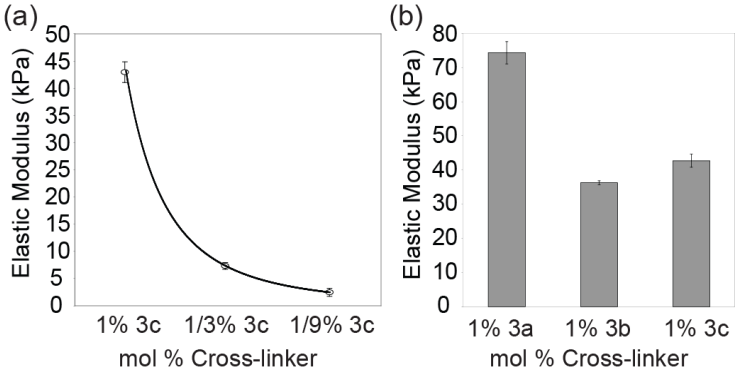


Fig. S2 (a) Elastic modulus of gels as a function of molar ratio between TAA cross-linker and acrylamide. TAA **3c** was used as a cross-linker. (b) Elastic modulus of gels as a function of cross-linker. A constant molar ratio between TAA and acrylamide was used.

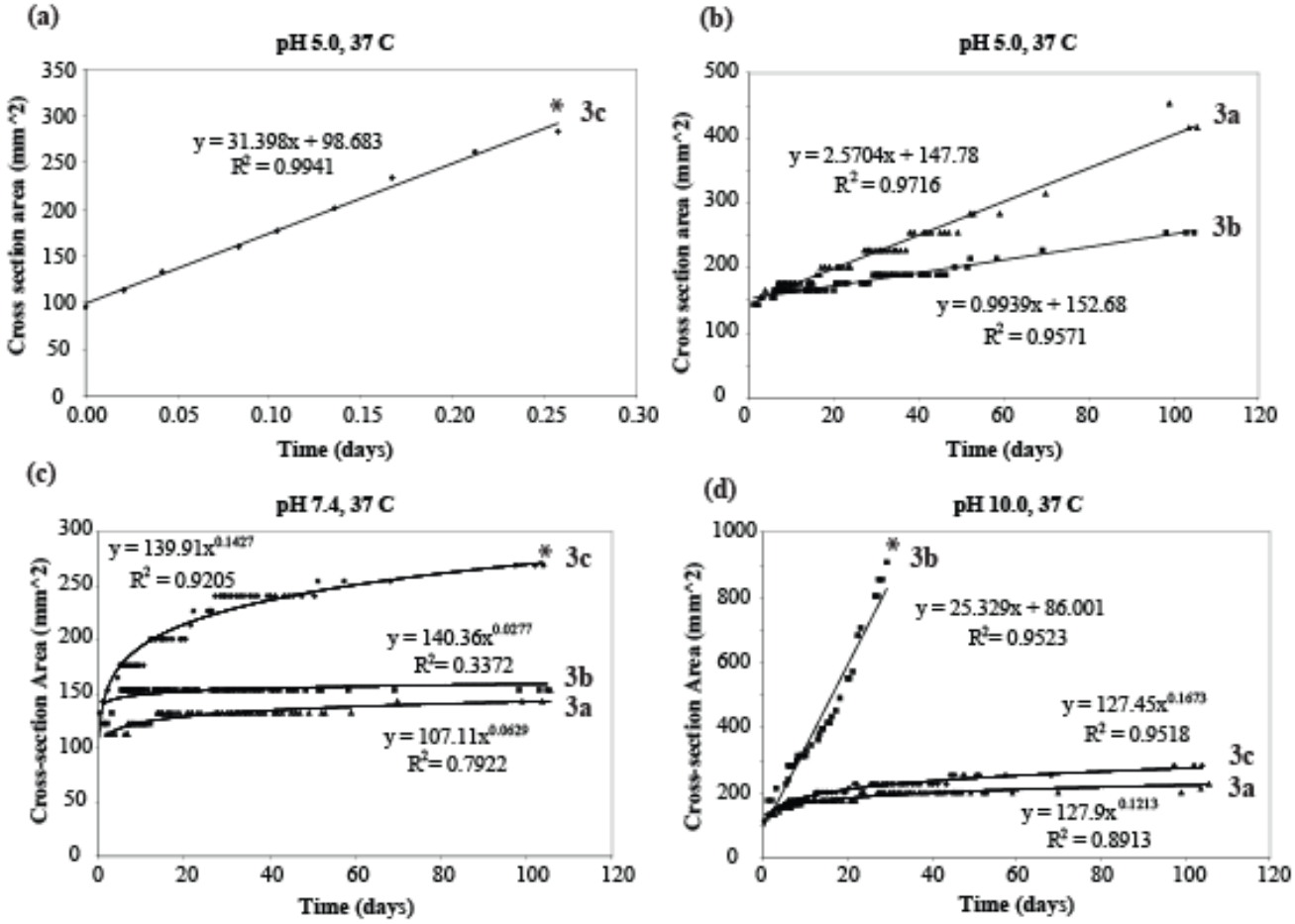


Fig. S3 Raw data for degradation of poly(acrylamide) hydrogel discs crosslinked with 1 mol % **3a**, **3b**, or **3c** at (a,b) pH 5.0, (c) pH 7.4, and (d) pH 10.0 at 37 °C. Disc diameters were measured over time and converted into surface area. * indicates last measurement taken before degradation was observed.

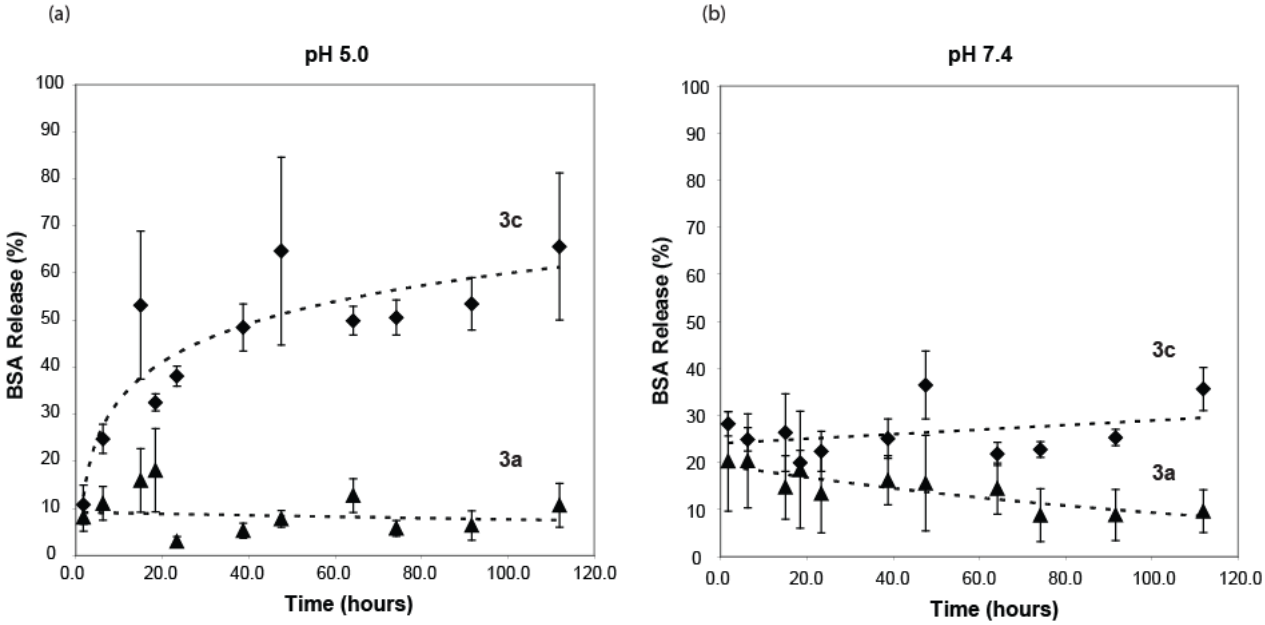


Fig. S4 The release percentage over time of bovine serum albumin (BSA) from polyacrylamide hydrogels cross-linked by TAAs **3a** or **3c**. Hydrogels cross-linked with 5 mol % TAA were incubated in PBS at 37.0 °C at (a) pH 5.0 and (b) pH 7.4.

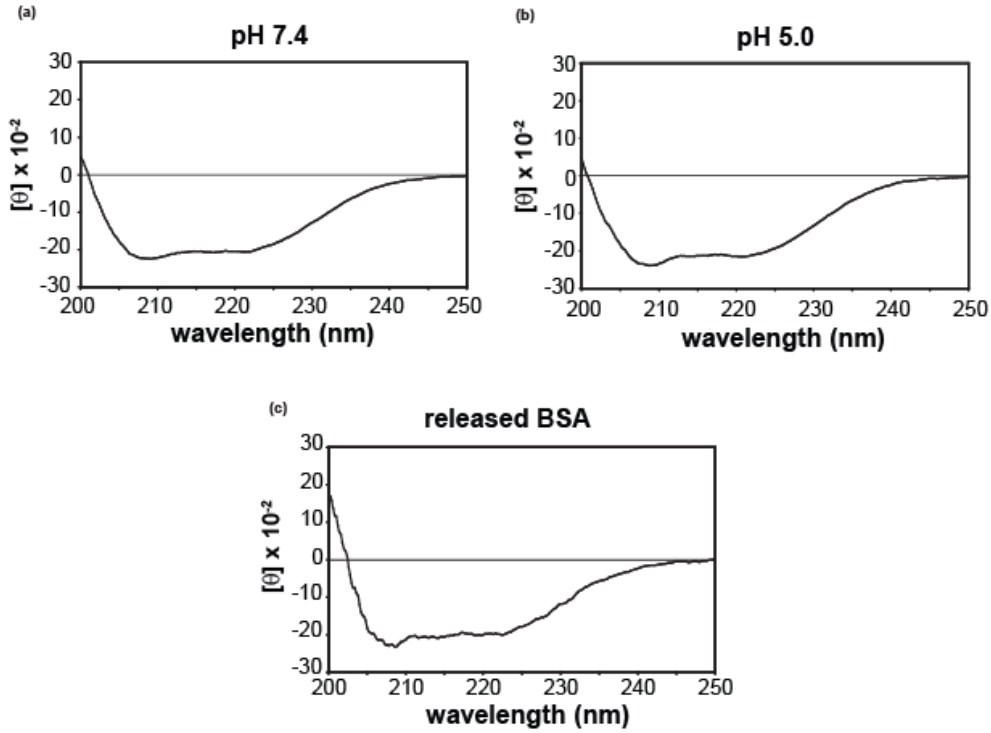


Fig. S5 Analysis of protein denaturation via circular dichroism. Spectra of commercial BSA in 100 mM PBS at (a) pH 7.4 and (b) pH 5.0. (c) Spectrum of BSA after being released from poly(acrylamide) hydrogel discs crosslinked with 5 mol % **3c** after 46 hours in pH 5.0 PBS.

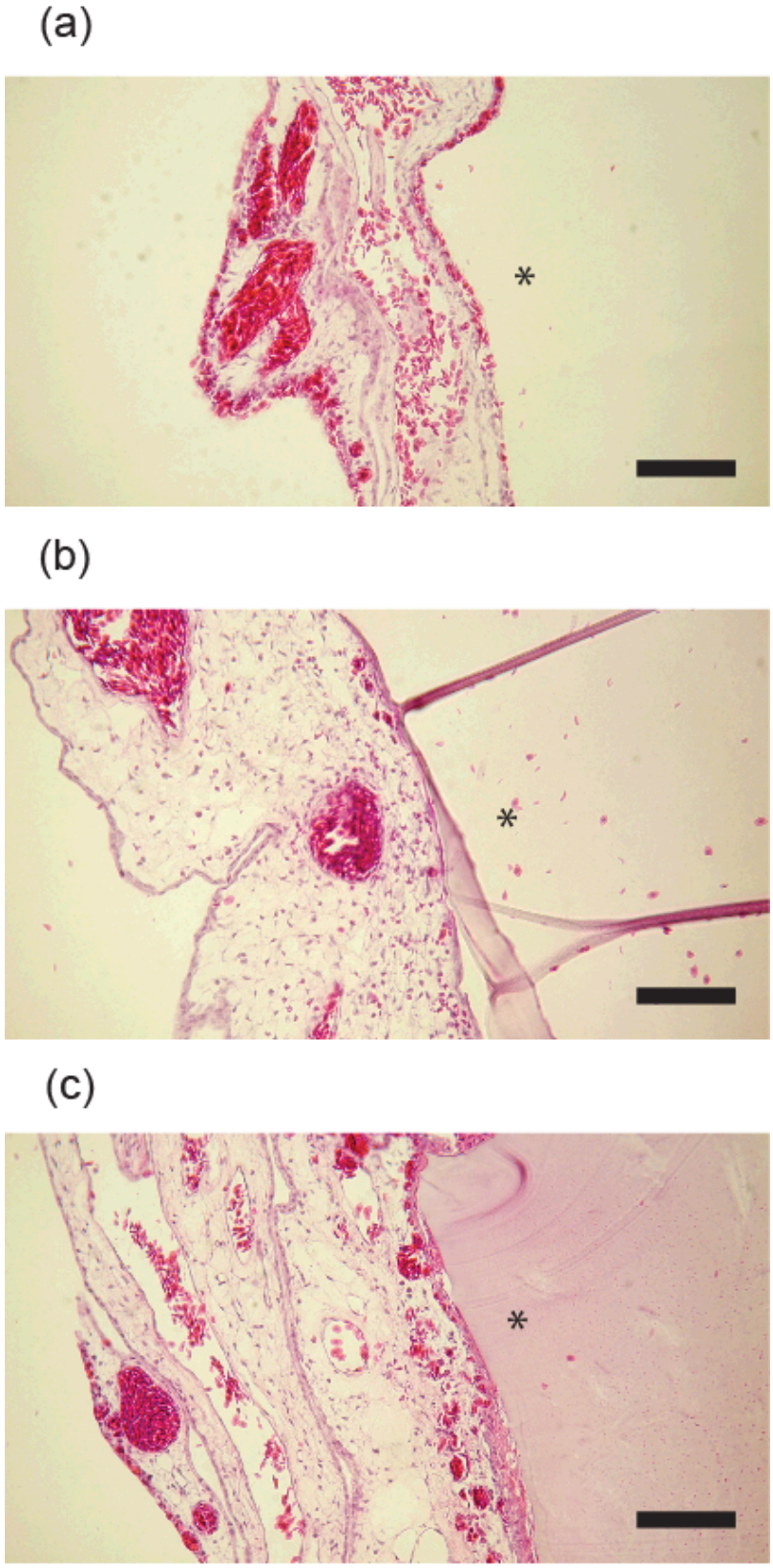


Fig. S6 Microscopic images of the cross-section of hydrogels (* indicates the hydrogel or the region where hydrogel was present) cross-linked by (a) TAA **3a**, (b) **3b**, or (c) **3c** and implanted within the chorioallantoic membranes (CAM). Hydrogels implanted in the CAMs resulted in a minimal inflammatory response. The cross-section was stained with hematoxylin and eosin stain. Scale bar equals 200 μm.

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